Lethality and synthetic lethality in the genome-wide metabolic network of *Escherichia coli*

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ABSTRACT

Recent genomic analyses on the cellular metabolic network show that reaction flux across enzymes are diverse and exhibit scale-free behavior in its distribution. Considering the roles played by main arteries or principal highways, one may guess that the reactions with larger fluxes are more likely to be lethal under the blockade of its catalyzing gene products or gene knockouts. However, we find, by *in silico* flux analysis, that the lethality rarely has correlations with the flux level owing to the widespread backup pathways innate in the genome-wide metabolism of *Escherichia coli*. Lethal reactions, of which the deletion generates cascading failure of following reactions up to the biomass reaction, are identified in terms of the Boolean network scheme as well as the flux balance analysis, where the avalanche size, the distribution of which follows a power law, turns out to be a useful measure of lethality. Even if the single deletion itself may not lead to the fatal results, simultaneous removal of two or more reactions can cause the failure of the biomass reaction, for which we adopt the term synthetic lethality as a metabolic homologue of the same situation in gene knockout studies. Identification of synthetic lethals in genome-wide metabolism provides a novel way to improve our understanding of essential biochemical reaction processes and their functionally redundant pathways, which is of potential applications to metabolic engineering.

Complex machinery of cellular metabolism occurring in a living organism makes up a part of autocatalytic network of biochemical reaction pathways. The reactions are catalyzed by enzymes, functional proteins, and/or cofactors which, again, are produced through the reaction network responsible for sustaining life. Though the network of intracellular reaction constitutes an intricate web of pathways interfering with each other, molecular level description of metabolism has been mainly pursued on the specific pathway basis, such as glycolysis/gluconeogenesis, citrate cycle, and other catabolic/anabolic reaction pathways. Only recently, advances in high-throughput experiments and the computing power incorporating diverse data sets collected in genomic research make it possible to construct cellular networks of metabolism, signal transduction, genetic regulations, and protein-protein interactions in genome-wide perspectives. At the same time, many quantitative theoretical methods including graph theories and other mathematical tools developed from diverse disciplines attract much attention to tackle the large-scale networks.

In the early graph-theoretic approaches to the metabolic network, attention has been paid to the so-called scale-free feature of topological structure [1], small-world-ness [2], modularity [3] and hierarchical organization [4]. Despite the immanent specificity in cellular functions of various organisms, the connectivity, or number of connections each node (metabolite or associated reactions) has, is generally far from homogeneous. In particular, this connectivity distribution of the metabolic network, as shared by many naturally occurring complex networks, follows a power law, meaning large deviations in spite of well defined average value. It is this context that borrows the term *scale-free* network, where hubs, nodes with large number of connections, play essential roles. When such hubs are removed or turned off, the whole system becomes vulnerable. Indeed, it was found [5] that, for the yeast protein interaction network, hub proteins are more likely to be lethal than the others.

In the framework of networks, metabolic reactions and participating metabolites can be mapped into alternating nodes, where the outward(inward) connections from a reaction node indicate that those metabolites are produced(consumed) as a result of the reaction. Once constructing a directed bipartite graph in this way, we calculate graph-theoretic quantities that characterize the global topology and give a clue to assessing lethality of metabolic reactions. Then, we study the putative correlations between the metabolic flux level and the lethality of each metabolic reaction using the flux balance analysis(FBA) [6]. Here, by lethal, we mean the organism could rarely synthesize the indispensable biomass, or the flux of the biomass reaction is significantly reduced when that reaction is blocked or removed from the network, mimicking gene knockout experiments. One of our main results, obviously counterintuitive, can be phrased as superhighway is no more lethal than sideways. It is related with the fact that the high-flux reactions have abundant bypasses or backup pathways.

We also introduce the Boolean network scheme, an idealization of the metabolic network as a wiring of binary logic gates to elucidate the pathway structure of the network

on the logical basis. Considering the knockout and consequent cascading failure in the metabolic reaction network as an avalanche, we investigate the distribution of avalanche sizes to find it a unique measure of lethality. The distribution also follows a power law with the characteristic exponent around 2.5, pervasive throughout disparate model systems having self-organized criticality [7].

In the latter part, we study the *synthetic lethality*, the effects of simultaneous multiple knockout. A pair of genes or reactions catalyzed by their gene products can be collectively lethal when they are removed simultaneously from the network, which manifests that the two reactions are responsible for the same function or pathway, or complementary in the sense that one takes place of the other otherwise silent. Synthetically lethal reaction pairs show strong correlations between lethality and their avalanche size, and are distributed over distinct pathways, reflecting deeper entanglement implicit in the global network.

Materials and Methods

We use, with minor curation, the recent revision of *in silico* model *E. coli* [8], which was obtained by searching databases, such as LIGAND (http://www.genome.jp/kegg/ligand.html), EcoCyc (http://www.ecocyc.org), TC-DB (http://tcdb.ucsd.edu/), and referring to updated literatures on sequence annotation [9]. To mimic random or targeted mutation strains, a specific reaction is removed from the network and the resultant metabolic capabilities are to be assessed. For this purpose, we introduce a single pivotal reaction, the biomass reaction, originally formulated as a linear combination of essential metabolic reactions giving rise to the growth of the organism [10]. Throughout the study, lethality of a certain reaction or corresponding gene products is determined by the flux of this biomass production, which is contingent to the ansatz of optimality that the selection pressure has imposed in the long history of evolution.

Metabolic Network as a Graph. The overall map of metabolic reactions we study is a bipartite graph, composed of two different types of nodes, 627 metabolites and their participating 1074 metabolic reactions including transport and exchange events. One type of nodes connect only to the other type of nodes in the networks[1]. Each link between a pair of a metabolite and a reaction is directed, reflecting the metabolite is either consumed (substrate) or produced (product) or both in reactions. Of the 1074 reactions, 254 reversible reactions are decomposed into two separate reactions catalyzed by the same enzyme. 627 distinct metabolites have either their intracellular or extracellular version or both, which sum into 761 distinct nodes of metabolites. Once the metabolic network is reconstructed as a graph, we quantify, by various numerical measures, the lethality of each node in the wild-type strain and compare them with those of knockout mutants.

[1]Depending upon the objectives, it can be projected to recover the single-mode metabolite network or reaction (enzyme) network.

Flux Balance Analysis (FBA). For each metabolite in the metabolic reaction network, dynamic flux balance condition on the concentration X_i can be written as

$$\frac{\partial X_i}{\partial t} = -\sum_{j=1}^{n} S_{ij}\phi_j \ , \tag{1}$$

where ϕ_j is the corresponding reaction rate (outward flux), and S_{ij} are stoichiometric coefficients involved in the metabolite $i \in \{1, 2, \cdots, m\}$ participating in the reaction $j \in \{1, 2, \cdots, n\}$ including transport and exchange reactions also. As an alternative to yet intractable kinetic models in genome-wide perspectives, mass conservation can be applied in the balanced state to give the stationary condition $\sum_j S_{ij}\phi_j = 0$ for all i. Stoichiometric matrix encodes the topology of the metabolic network and gives how the complex reactions are entangled with one another. Once the stoichiometric coefficients are given for all reactions, we have, in general, under-determined situation($m < n$) where huge degeneracy in the null space of Eq. (1) is unavoidable. Actually, this multiplicity in the feasible metabolic state can be considered a manifestation of the capability of a metabolic genotype. It makes physiological sense in that cells are expected to adapt themselves responding to different stresses and growth conditions. Among those feasible metabolic phenotypes, FBA assumes the existence of optimized point(s) of a certain objective function, called biomass reaction or growth flux, by utilizing further constraints based on the thermodynamic irreversibility of reactions.

The Boolean Network. Metabolic reactions or genetic switches are seldom turned on or off. Instead, they can change by some fold, either up- or down-regulated, to make a physiological payoff. As a first approximation, Boolean idealization has long been introduced to reconstruct the genetic regulatory network as a directed graph [11] and was recently adopted to model the metabolic network [12]. In Boolean reconstruction of the metabolic network, each node in the graph is replaced with binary logic gate AND or OR. A metabolite would not cease to be existent in the network until all the reactions having that metabolite as a product are blocked, while a reaction does not take place any more with only a single absence out of substrate metabolites, which renders the metabolites Boolean disjunction, OR, and reactions conjunction, AND as exemplified in Fig. 1. With all nodes given an initial condition, we iteratively relax the network till the network is settled in a fixed point.

Cumulative Lethality Score. As a way to reveal the correlation between avalanche size and lethality, we use the index, cumulative lethality score (CLS), the original version of which was introduced in the context of protein essentiality prediction [13]. Once a measure of lethality, say the load of each node, is proposed, we can make a serial list of reactions assorted in descending order of the proposed lethality measure. With the lethality criteria determined through the viability under the single deletion of each reaction, we can assign a binary lethality score, one or zero, into each reaction depending on

whether it is lethal or not. Summing up the binary scores from the first rank to the jth rank in the value of the proposed quantity, say, flux level, we get the CLS, say, $L(j)$. If the proposed quantity is positively(negatively) correlated with the lethality, *i.e.* reactions holding high ranks are dominated by the binary lethality score one(zero), the normalized CLS manifests itself as a convex(concave) curve. Otherwise, if it is a random sequence of zero's and one's, $L(j)$ is given by a straight line. That is, the more correlated is the measure with the lethality, the higher curvature $L(j)$ develops.

Results and Discussion

Flux Level versus Lethality. Under aerobic condition with glucose as a unique external carbon source[2], we identify 210 (19.7%) lethal reactions out of 1064, the single blockade of which suffocates the biomass production. It is similar in fraction to the essential fraction of *S. cerevisiae* genome, 18.7% [15, 16, 17].

The flux distribution in the wild-type metabolic network follows a power-law in Pareto's form, $P(\phi) \sim (\phi + \phi_0)^{-\alpha}$. Metabolic traffic is concentrated along a few 'superhighway' reactions, while the vast majority of reactions are in charge of only a small flux [18]. In the meantime, inspired by the roles the main arteries, principal roads, or backbones play in blood circulation, transportation, or data communications, we examine the possibility that the flux level should reflect the lethality of each reaction. Fig. 2 shows that the plausible correspondence between flux level and lethality is a mere conjecture to prove not true. In other words, there is no correlation at all between those quantities, and high flux itself has nothing to do with the lethality of a reaction, which obviously contradicts our intuition. We also investigate the flux redistribution profile upon deletion of a high-flux reaction. Reaction fluxes are redistributed either locally or globally. Here, by local, we mean the case that very few reactions, having almost zero flux in the wild type, fully take over the flux of the reaction deleted. However, in the global redistribution, the flux of the removed reaction is shared over a large number of reactions to keep optimal biomass production.

Avalanche Size versus Lethality. In the Boolean reconstruction of the *E. coli* metabolic network, 41 lethal reactions are identified, which are all lethal in FBA also. It is no wonder, if we consider that lethality in binary scheme is minimal and more stringent than in the weighted version of FBA. To quantify the effect of a single-node deletion, we define the avalanche size of each reaction, in the Boolean scheme, as the number of reactions subsequently turned off on account of targeted removal of that reaction.

As shown in the inset of Fig. 3, the avalanche size distribution for *E. coli* metabolic

[2]Throughout the numerical experiments, acetate(ace), alpha-ketoglutarate(akg), glucose(glc), glycerol(glyc), lactate(lac-L, lac-D), malate(mal-L), pyruvate(pyr), and succinate(succ) are used as the carbon sources, for each of which, we also control the oxygen uptake rate. Though the lethality of a reaction does depend on which are used as carbon sources, across which it is largely preserved. For details of the nutrient-dependent results, see *Supporting Information*.

network also displays a power law behavior with a fat tail, implying there exist a few reactions whose deletion triggers a large destructive avalanche cascade in metabolic reaction. In mathematical aspects, at criticality, there exists an infinite spanning cluster enough to reach the ultimate destination, biomass. Thus, avalanche size could be useful to find lethal reaction. Indeed, the reaction which generates a bigger avalanche size is more likely to be lethal as seen in the CLS plot (main panel of Fig. 3).

One may want to check the possibility that nearer nodes to the biomass reaction should be liable to be lethal. However, of the 374 distinct reactions that produce the substrates of biomass reaction, only 13 of them are lethal in the Boolean scheme, making the identification of lethal reactions nontrivial—proximity to the biomass reaction has nearly nothing to do with the lethality of a reaction.

The notion of the avalanche in the network can be extended to the FBA scheme, where the avalanche size of a reaction is defined as the number of reactions whose flux levels under its knockout differ from those in the wild type. Actually, we used three different criteria of the avalanche: (i) reactions are newly turned on or off, (ii) flux level change exceeds an arbitrary cutoff value, and (iii) fractional changes in flux exceeds the cutoff value. There is, however, little difference among the different counting schemes. Fig. 4, drawn by using the first criterion above, reveals the absence of correlation between the flux level and the avalanche size both in the Boolean scheme and in FBA, which is consistent with the fact that flux level is irrelevant to assessing lethality.(Fig. 2)

Due to the small-world-ness of the complex network, local perturbations are liable to propagate to the whole network leading to the sharing of load, which underlies the system-wide high flexibility. At the same time, because of the scale-free-ness, avalanche cascade can either be long-ranged by making a large number of nodes bear parts of the 'expenses' or be absorbed at a short distance from the source of perturbation, depending on the detailed functional characteristics of the reaction. In other words, the response to single deletion perturbation of a single reaction are too diverse to definitely predict how they would be, and so they can be predicted in a probabilistic way [19]. Identification of lethal reactions in metabolic network can be viewed in the same footing. The effects of a node removal or a gene deletion are largely negligible as a whole, which is manifested by the dominance of nonlethal reactions. However, even if a reaction is not lethal, its potential damage to the network varies. It is this insufficiency in lethality assessing that raises the need for the knowledge of synthetic lethals in the next section.

Synthetic Lethality in Metabolic Network. The close genetic relationships between genes which underlie the functional buffering has been associated with the notion of synthetic lethality. It has been assessed in a high-throughput manner by the synthetic genetic array(SGA) analysis [15]. Likewise, analysis of multiple-deletion mutants in genome-scale metabolic network may shed light on novel topological features of backup pathways leading to the robustness. In the restricted level of metabolism, such relationships can be revealed more clearly by performing the double reaction knockout experiments,

which can be easily performed *in silico*. Furthermore, such metabolically synthetic lethal pairs identified allow us to track the backup pathways explicitly and to visualize the precise microscopic origin from which the metabolic flexibility arises.

When the glucose is used as a carbon source in aerobic condition, 55 synthetic lethal pairs are identified (Table I). The relatively small number of synthetic lethal pairs suggests the low density of backup pathways for a given specific condition in the metabolic network and is in accordance with the case of the yeast [20]. Among these, 33 (60%) pairs are involved in the same subdivision of the reaction categories. As expected, most of those homofunctional synthetic lethals usually work as the 'simple' backup pathway: One of the pair is not used in the wild type at all but it almost completely takes over the flux of the blocked reaction (64%) as depicted in Fig. 5(a). Interestingly, the homofunctional synthetic lethals of the other type, for which both the reactions are operational in the wild type, are mostly involved in the two subsystems of the pentose phosphate cycle and threonine-lysine metabolism. Excluding these particular cases, 91% of homofunctional synthetic lethals are simple, while, for the other 22 heterofunctional synthetic lethals, only 9 (41%) of them are the simple backup pathways. In total, 25 pairs were both operational in the wild type and the remaining 30 are paired in one used and one unused in the wild type.

We also study the synthetic lethality in the Boolean scheme. In the Boolean scheme, 37 pairs of synthetic-lethal doublets are identified in addition to 41 lethal singlets. Then, we focus on the functional categories lethal reactions belong to. In Fig. 6, it's noteworthy that cell envelope biosynthesis dominates (78%) all the other reaction categories under single knockouts in the Boolean scheme, which means lack of backup pathways, while the synthetic lethals are scattered throughout diverse functional categories.

Conserved across both the network scheme is the proximity of the two reactions constituting a synthetic-lethal pair. Fig. 5(c) illustrates the distance distribution for the synthetic-lethal reaction pairs, around 65% of which are just two step apart sharing common metabolites, and over 95% of which are within four steps. Considering that an arbitrary pair of reactions are connected in two steps with the probability 30%, synthetic lethals are highly lumped with each other in the metabolic reaction network. Whether the analytical scheme is Boolean or FBA, long-ranged complicity of synthetic lethals through the intermediary of a 'filamentary' single pathway like in Fig. 5(b) comprises only a small fraction($<$5%) of synthetic-lethal pairs, and is rather an exceptional case.

Another important outcome regarding synthetic lethality is already shown in Fig. 3, where we measure the avalanche size of the synthetically lethal doublets and triplets in addition to that of the singlets. Synthetic-lethal multiplets give rise to even higher correlation of their avalanche size with the lethality. In effect, robustness in metabolic network stems from redundancy in branched and parallel pathways. Conversely, lack of reaction pathways, whether it is due to the unique biochemical nature or to the defects in pathway database, lead to vulnerability. Hence, the more we know about a reaction pathway, the less probable it should contain lethal reactions. In particular, we

cannot completely rule out the latter possibility: Accumulated bias in molecular biology research, if any, might be crucial to our result that generic pathways across the species, such as the citrate cycle or the glycolysis pathways, have very few lethal reactions (Fig. 6). However, at least for *E. coli*, one of the best known bacteria yet studied, there are no good reasons to suspect such a bias. Moreover, our analytical results are compatible with the fact that a wide-spread strategy of antimicrobials is acting against cell wall synthesis(fosfomycin, cycloserine) or integrity(lysozyme). Rather to be supposed is that the more important a reaction is, the better facilitated its backup pathways have been during evolution.

Summary and Outlook

Systematic deletion study in a genome-wide view of model organisms help reveal the organizing principles of the metabolic network and may shed light on how the selection has been embodied at the network levels, and especially the recent controversy surrounding the causes and evolution of the enzyme dispensability [20]. As an index quantifying lethality in the graph-reconstructed metabolic reaction network, we propose the avalanche size of each reaction, the number of 'dead' reactions due to the knockout of that reaction or its related gene products to show an even more remarkable interdependence than various measures yet proposed. By identifying synthetic lethals or lethal multiplets in the genome-scale metabolic network under controlled environments, we see the emergence of new phenotypes supported by rich backup pathways, which is shared by diverse levels of biological networks. Studies on multiple deletion mutations in metabolomic interaction network can also be applied to natural metabolomic variations, reminiscent of single-nucleotide polymorphism, giving rise to practical buffering or phenotypic robustness under targeted mutations [15, 21, 22]. Furthermore, if we incorporate network analyses on metabolism with the gene-protein-reaction associations by including the other sectors of intra- and inter-cellular networks, it can be used in designing new microorganismal strains on the computer truly beyond the reductionist perspectives.

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Figures & Tables

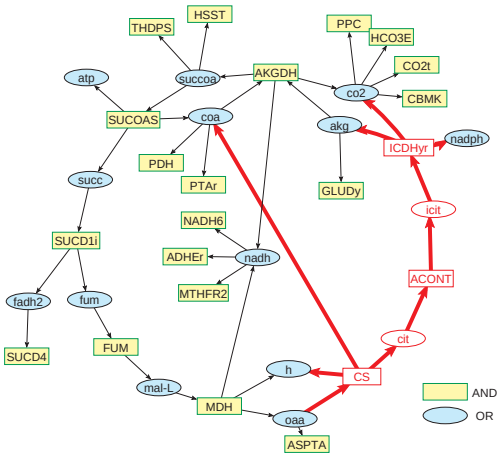

Figure 1: A subgraph of the citrate cycle in *E. coli* metabolic network. In Boolean scheme, metabolites(ellipses) are treated as Boolean disjunction(OR), while the reactions(rectangles) as conjunction(AND). If this graph were isolated, though actually not the case, from the other reactions and metabolites, the metabolite `coa` would be no more supplied only when both the reactions `SUCOAS` and `CS` are blocked. On the other hand, the reaction `AKGDH` would not be operational when either of the metabolites `coa` or `akg` is knocked out. In this hypothetical subnetwork severed from the other part, bold red arrows indicate blocked reaction paths due to the knockout of the reaction `CS`, which has the avalanche size three, number of red rectangles.

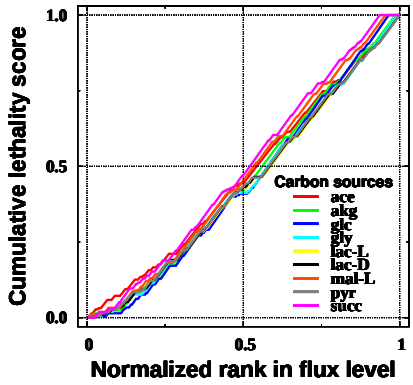

Figure 2: Normalized cumulative lethality score with respect to the wild-type flux levels grown on distinct carbon sources. Flux level shows no conspicuous convexity, if any, implying they cannot be a lethality measure.

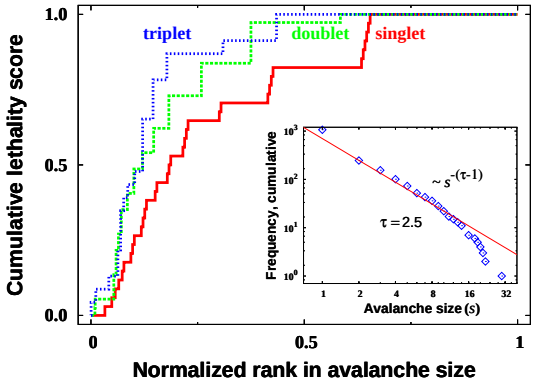

Figure 3: Normalized cumulative lethality scores drawn for the avalanche size of single and multiple deletion in the Boolean version of the *E. coli* metabolic network. Inset: Boolean avalanche size distribution under single targeted deletion of each reaction follows a power-law with the (noncumulative) exponent 2.5.

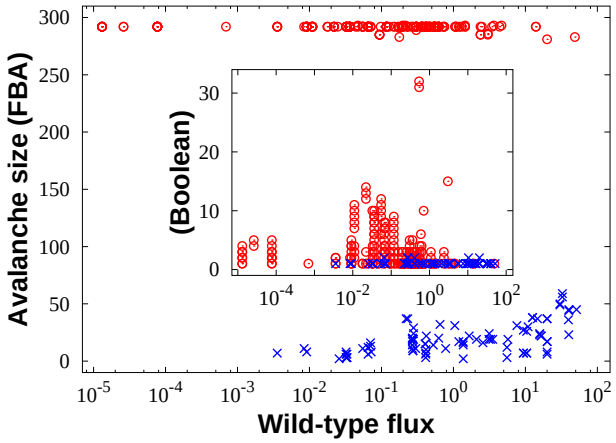

Figure 4: Lethal (red circles) and nonlethal (blue crosses) reactions in the avalanche size and the wild-type flux level determined by FBA (main panel) and the Boolean scheme (inset). In both the FBA and Boolean schemes, lethal reactions have larger avalanche size and are dispersed along wide range of flux level at the same time. Here, only the reactions being turned on in the wild-type strain are plotted.

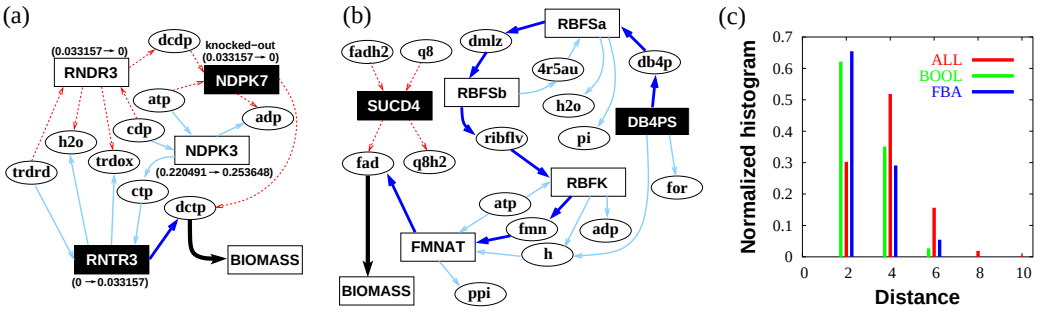

Figure 5: (a) As a consequence of NDPK7(nucleoside-diphosphate kinase, ATP:dCDP) removal, the reaction RNDR3(ribonucleoside-diphosphate reductase) is also blocked(dotted red lines), but RNTR3(ribonucleoside-triphosphate reductase) is newly turned on(solid (sky-)blue lines), with NDPK3(nucleoside-diphosphate kinase, ATP:CDP) flowing more flux to make up for the growth flux. Here, only three reactions are retuned, two of which depicted by filled blocks constitute a synthetic-lethal pair in both the Boolean and FBA schemes. The flux changes are shown in units of mm/g DW-hr. (b) *E. coli* can subsist without the reaction SUCD4, but, in the absence of SUCD4, all the reactions DB4PS, RBFSa, RBFSb, RBFK, and FMNAT become lethal. In particular, DB4PS has a 'long-range correlation' with SUCD4 along with the intermediary lethal reactions. Other parts of the network that the avalanche cascade does not reach are omitted for brevity. (c) Synthetic lethals tend to be closer neighbors than arbitrary pairs of reactions. Here, ALL refers to the distances for all pairs of reactions, while BOOL(FBA) stands for the Boolean(FBA) synthetic-lethal doublets. In measuring distances, only the connection is taken into account, the direction of edges being disregarded.

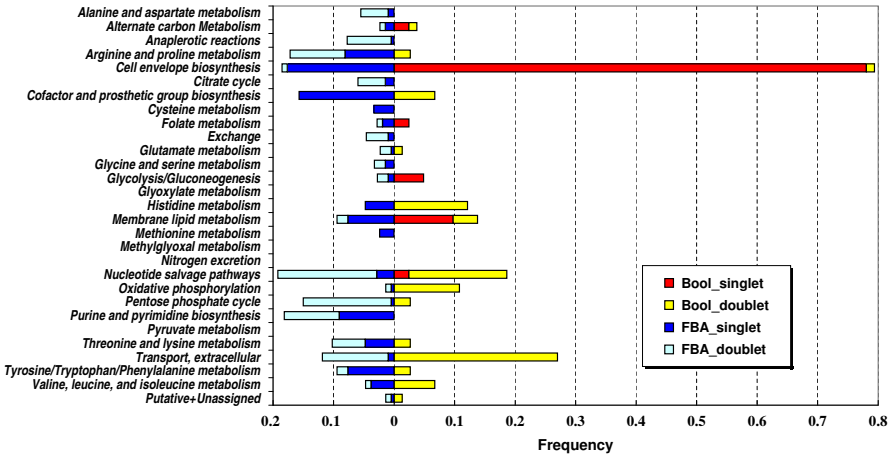

Figure 6: Classification of lethal and synthetic-lethal reactions according to the functional categories. Frequencies are normalized by total number of lethal singlets and doublets in both the Boolean and FBA scheme, respectively.

Reaction I	Reaction II	Category I	Category II	$\phi_{\text{I, wild}}$	$\phi_{\text{II, wild}}$
ASNS2	ASNS	Alanine, aspartate metabolism	"	0.298933	0.000000
ALAR	ALARi	Alanine, aspartate metabolism	"	0.072057	0.000000
PPC	MALS	Anaplerotic reactions	"	3.519873	0.000000
PPC	ICL	Anaplerotic reactions	"	3.519873	0.000000
DKMPPD2	DKMPPD	Arginine, Proline Metabolism	"	0.009138	0.000000
ORNDC	ARGDC	Arginine, Proline Metabolism	"	0.054826	0.000000
ORNDC	AGMT	Arginine, Proline Metabolism	"	0.054826	0.000000
GLUDy	GLUSy	Glutamate metabolism	"	-10.86474	0.000000
KAS15	KAS14	Membrane Lipid Metabolism	"	0.41942	0.000000
ADK	ADK3	Nucleotide Salvage Pathways	"	3.284564	0.000000
RNTR2	RNDR2	Nucleotide Salvage Pathways	"	0.033157	0.000000
RNTR2	NDPK5	Nucleotide Salvage Pathways	"	0.033157	0.000000
RNTR	NDPK8	Nucleotide Salvage Pathways	"	0.032243	0.000000
RNDR3	RNTR3	Nucleotide Salvage Pathways	"	0.033157	0.000000
NDPK7	RNTR3	Nucleotide Salvage Pathways	"	0.033157	0.000000
NDPK	ADK3	Nucleotide Salvage Pathways	"	1.045572	0.000000
GARFT	GART	Purine, Pyrimidine Biosynthesis	"	0.623856	0.000000
DHORD5	DHORD2	Purine, Pyrimidine Biosynthesis	"	0.411327	0.000000
O2t	SUCCt2b	Transport, Extracellular	"	20.00000	0.000000
PIt2r	PIabc	Transport, Extracellular	"	1.190038	0.000000
TRPS3	TRPS	Tyrosine, Tryptophan, Phenylalanine Metabolism	"	0.070490	0.000000
RNDR	RNTR	Nucleotide Salvage Pathways	"	0.269780	0.032243
ADK	NDPK	Nucleotide Salvage Pathways	"	3.284564	1.045572
RPE	TKT2	Pentose Phosphate Cycle	"	5.716916	2.573754
RPE	TKT	Pentose Phosphate Cycle	"	5.716916	3.143163
RPE	TALA	Pentose Phosphate Cycle	"	5.716916	3.110267
TALA	TKT2	Pentose Phosphate Cycle	"	3.110267	2.573754
TALA	TKT	Pentose Phosphate Cycle	"	3.110267	3.143163
TKT	TKT2	Pentose Phosphate Cycle	"	3.143163	2.573754
GND	TKT2	Pentose Phosphate Cycle	"	10.42057	2.573754
GND	RPE	Pentose Phosphate Cycle	"	10.42057	5.716916
THRAr	THRS	Threonine, Lysine Metabolism	"	-0.26978	0.405103
THRAr	HSK	Threonine, Lysine Metabolism	"	-0.26978	0.405103
ORNDC	UREAt	Arginine, Proline Metabolism	Transport, Extracellular	0.054826	0.000000
ORNDC	EX_urea	Arginine, Proline Metabolism	Exchange	0.054826	0.000000
GALUi	GALU	Cell Envelope Biosynthesis	Alternate Carbon Metabolism	0.025847	0.000000
FUM	SUCCt2b	Citrate Cycle (TCA)	Transport, Extracellular	1.368573	0.000000
FRD2	DHORD2	Citrate Cycle (TCA)	Purine, Pyrimidine Biosynthesis	0.411327	0.000000
MTHFD	GART	Folate Metabolism	Purine, Pyrimidine Biosynthesis	1.365196	0.000000
MTHFC	GART	Folate Metabolism	Purine, Pyrimidine Biosynthesis	1.365196	0.000000
CBMK	CBPS	Putative	Arginine, Proline Metabolism	0.77814	0.000000
VALTA	VPAMT	Valine, leucine, isoleucine metabolism	Alanine, aspartate metabolism	-0.524765	0.000000
SUCD1i	PPC	Citrate Cycle (TCA)	Anaplerotic reactions	0.414887	3.519873
FUM	PPC	Citrate Cycle (TCA)	Anaplerotic reactions	1.368573	3.519873
SUCD4	PPC	Oxidative phosphorylation	Anaplerotic reactions	0.414887	3.519873
GARFT	MTHFD	Purine, Pyrimidine Biosynthesis	Folate Metabolism	0.623856	1.365196
GARFT	MTHFC	Purine, Pyrimidine Biosynthesis	Folate Metabolism	0.623856	1.365196
THRS	GHMT2	Threonine, Lysine Metabolism	Glycine, Serine Metabolism	0.405103	1.653370
HSK	GHMT2	Threonine, Lysine Metabolism	Glycine, Serine Metabolism	0.405103	1.653370
O2t	PPC	Transport, Extracellular	Anaplerotic reactions	20.00000	3.519873
O2t	DKMPPD2	Transport, Extracellular	Arginine, Proline Metabolism	20.00000	0.009138
O2t	FRD2	Transport, Extracellular	Citrate Cycle (TCA)	20.00000	0.411327
O2t	GAPD	Transport, Extracellular	Glycolysis/Gluconeogenesis	20.00000	32.61301
O2t	PGK	Transport, Extracellular	Glycolysis/Gluconeogenesis	20.00000	-32.61301
O2t	DHORD5	Transport, Extracellular	Purine, Pyrimidine Biosynthesis	20.00000	0.411327

Table 1: List of synthetic-lethal reactions. Upper(lower) two cells include synthetic lethals belonging to the same(distinct) functional categories, and the wild-type flux of each reaction is given in units of mm/g DW-hr.